

What is claimed is:

*Sub
Rev 5*

1. A method of detecting the presence of one or more analytes (I_1C_A, \dots, I_nC_A) in a sample, the method comprising:
 - a) contacting a sample with a set of binding immunoreaction component-pairing component member complexes ($I_1C_B-P_1, \dots, I_nC_B-P_x$), wherein the set comprises at least one binding immunoreaction component-pairing component member complex for each analyte to be detected, producing immunoreaction complexes ($I_1C_A-I_1C_B-P_1, \dots, I_nC_A-I_nC_B-P_x$) of the analytes in the sample and their respective binding immunoreaction component-first pairing component member complexes;
 - b) contacting the complexes formed in step (a) with an active electronic matrix, wherein the active electronic matrix comprises at least one set of test sites, wherein the set comprises at least one test site comprising a complementary pairing component member attached to the test site's permeation layer for each pairing component member of in the set of binding immunoreaction component-pairing component member complexes of step (a);
 - c) electronically biasing, concurrently or sequentially, the set of test sites to which the complementary pairing components are attached, whereby the pairing component members on the immunoreaction complexes and on the test sites are allowed to bind, producing a bound immunoreaction complexes at the biased test sites;
 - d) detecting the presence of the bound immunoreaction complexes at the test sites.
2. The method of claim 1 wherein at least one analyte is an epitope bearing moiety.
- 25 3. The method of claim 1 wherein at least one analyte is a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.
4. The method of claim 1 wherein at least one binding immunoreaction component

in the set of binding immunoreaction component-pairing component member complexes is an epitope bearing moiety.

5. The method of claim 1 wherein at least one binding immunoreaction component in the set of binding immunoreaction component-pairing component member complexes is a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.
6. The method of claim 1 wherein at least one of the pairing components is p-RNA.
- 10 7. The method of claim 1 wherein at least one of the pairing components is CNA.
8. The method of claim 1 wherein at least one of the pairing components is DNA.
9. The method of claim 1 wherein at least one of the pairing components is RNA.
10. The method of claim 1 wherein at least one of the binding immunoreaction components is attached to its respective pairing component member through a covalent bond.
- 15 11. The method of claim 1 wherein at least one of the binding immunoreaction components is attached to its respective pairing component member through a non-covalent interaction.
12. The method of claim 11 wherein the non-covalent interaction is selected from the group consisting of a biotin-streptavidin interaction and a biotin-avidin interaction.
- 20 13. The method of claim 1 wherein at least one complementary pairing component member is attached to its test site through a covalent bond to the permeation layer.
14. The method of claim 1 wherein at least one complementary pairing component member is attached to the test site through a non-covalent interaction with a moiety in the permeation layer.
- 25 15. The method of claim 14 wherein the non-covalent interaction is selected from the group consisting of a biotin-streptavidin interaction and a biotin-avidin interaction.

16. The method of claim 1 wherein one analyte (I_1C_A) is detected.
17. The method of claim 1 wherein at least two analytes (I_1C_A, I_2C_A) are detected.
18. The method of claim 1 wherein at least three analytes (I_1C_A, I_2C_A, I_3C_A) are detected.
- 5 19. The method of claim 1 wherein at least four analytes ($I_1C_A, I_2C_A, I_3C_A, I_4C_A$) are detected.
20. The method of claim 1 wherein at least five analytes ($I_1C_A, I_2C_A, I_3C_A, I_4C_A, I_5C_A$) are detected.
21. The method of claim 1 wherein the active electronic matrix array comprises at 10 least a second set of test sites, wherein each set comprises at least one test site comprising a complementary pairing component member attached to the test site's permeation layer for each pairing component member of in the set of binding immunoreaction component-pairing component member complexes of step (a), wherein the second set of test sites is not electronically biased in step (c).
- 15 22. The method of claim 21 further comprising repeating steps (a) through (c) with at least one additional sample, wherein in step (c) of each repeat cycle, for each sample, a set of test sites which was not biased during step (c) for any of the other samples is biased so that the pairing component members on the immunoreaction complexes and on the biased test site are allowed to bind, producing bound 20 immunoreaction complexes on the biased test sites for that sample.
23. The method of claim 22 wherein two additional samples are processed so as to produce bound immunoreaction complexes for each sample.
24. The method of claim 22 wherein three additional samples are processed so as to produce bound immunoreaction complexes for each sample.
- 25 25. The method of claim 22 wherein four additional samples are processed so as to produce bound immunoreaction complexes for each sample.
26. The method of claim 22 wherein at least nine additional samples are processed so as to produce bound immunoreaction complexes for each sample.
27. The method of claim 1 further comprising contacting the sample with at least one

labeled analyte standard, wherein the labeled analyte standard comprises a detectable moiety and a moiety selected from the group consisting of the analyte and an immunochemical analog of the analyte, wherein the detection of the presence of the bound immunoreaction complex containing the analyte in step (d) is by detecting the decrease in the amount of the immunoreaction complex containing the labeled analyte standard bound at the test site.

5

28. The method of claim 27 wherein the labeled analyte standard comprises the analyte.

29. The method of claim 27 wherein the analyte is an antibody, and the labeled 10 analyte standard comprises an analog of the analyte comprising an isoidiotypic binding site, wherein the analyte analog is selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

15 30. The method of claim 27 wherein the analyte is an antigen, and the labeled analyte standard comprises an analog of the analyte comprising a structural domain selected from the group consisting of: an epitope of the antigen recognized by the binding immunoreaction component which binds the analyte, and a structural moiety which is anti-idiotypic to the binding site of the binding immunoreaction 20 component which binds the analyte.

31. The method of claim 27 wherein the labeled analyte standard comprises a detectable moiety selected from the group consisting of: fluorophores, chromophores, biotin, avidin, streptavidin, chemiluminescent agents, enzymes, gold particles, magnetic beads, metal chelates, radioisotopes, other antibodies, and 25 nanoparticles.

32. The method of claim 27 wherein the labeled analyte standard comprises a fluorophore selected from the group consisting of: BODIPY_{630/650} X-SE, Texas Red X-SE, or BODIPY TRX-SE, Cy-dyes, fluorescein, rhodamine, phycoerythrin, Lissamine, and coumarin.

33. The method of claim 1 further comprising allowing one or more labeling immunoreaction components to bind to one or more of the analytes to be detected, wherein at least one of the labeling immunoreaction components comprises a detectable moiety, and wherein the detection of the presence of bound immunoreaction complexes at the test sites in step (d) is by the detection of the labeling immunoreaction components bound to the analytes in the bound immunoreaction complexes.

5 34. The method of claim 33 wherein at least one of the labeling immunoreaction components comprises a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

10 35. The method of claim 33 wherein at least one of the labeling immunoreaction components comprises a moiety selected from the group consisting of biotin, avidin, and streptavidin.

15 36. The method of claim 33 wherein at least one of the labeling immunoreaction components comprises a detectable moiety fluorophores, chromophores, biotin, avidin, streptavidin, chemiluminescent agents, enzymes, gold particles, magnetic beads, metal chelates, radioisotopes, other antibodies, and nanoparticles.

20 37. The method of claim 33 wherein at least one of the labeling immunoreaction components comprises a fluorophore selected from the group consisting of: BODIPY_{630/650} X-SE, Texas Red X-SE, or BODIPY TRX-SE, Cy-dyes, fluorescein, rhodamine, phycoerythrin, Lissamine, and coumarin.

38. The method of claim 33 wherein at least one of the labeling immunoreaction components binds to more than one analyte in the sample.

25 39. The method of claim 33 wherein at least one of the labeling immunoreaction components is a dye with an affinity for one or more of the analytes to be detected greater than its affinity for any other component of the immunoreaction complex or the permeation layer of the active electronic matrix.

40. The method of claim 33 wherein at least one of the labeling immunoreaction components is contacted with the sample in step (a).

41. The method of claim 33 wherein at least one of the labeling immunoreaction components is contacted with the immobilized immunoreaction complexes produced in step (c).

5 42. The method of claim 41 further comprising electronically biasing one or more sites containing immobilized immunoreaction complexes while contacting the at least one labeling immunoreaction component with the immobilized immunoreaction complexes.

10 43. A method for detecting one or more analytes (I_1C_A, \dots, I_nC_A) in a sample, the method comprising:

a) contacting with an active electronic matrix array, either concurrently or sequentially, a set of binding immunoreaction component-pairing component member complexes ($I_1C_B-P_1, \dots, I_nC_B-P_x$), the set comprising a binding immunoreaction component-pairing component member complex for each analyte to be detected, wherein the active electronic matrix array comprises at least one set of test sites, the set consisting of at least one test site comprising a complementary pairing component member for each binding immunoreaction component-pairing component member complex;

15 b) electronically biasing, either concurrently or sequentially, each test site to which one of the binding immunoreaction component-pairing component member complexes is to be attached, so that the pairing component members are allowed to selectively bind, thus producing a set of attached binding immunoreaction component-pairing component member complexes ($I_1C_B-P_1-P_1', \dots, I_nC_B-P_x-P_x'$) bound to the set of test sites;

20 c) contacting the sample with the active electronic matrix array, and incubating the sample with the array under conditions suitable for the binding of the analytes in the sample with the immobilized binding

25

immunoreaction components, thereby producing immobilized immunoreaction complexes at one or more test sites;

d) detecting the presence of bound analyte in the immobilized immunoreaction complexes at the test sites.

5

44. The method of claim 43 wherein one or more analyte is an epitope bearing moiety.

45. The method of claim 43 wherein one or more analyte is a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

10 46. The method of claim 43 wherein one or more binding immunoreaction component in the set of binding immunoreaction component-pairing component member complexes is an epitope bearing moiety.

15 47. The method of claim 43 wherein one or more binding immunoreaction component in the set of binding immunoreaction component-pairing component member complexes is an epitope bearing moiety is a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

20 48. The method of claim 43 wherein at least one pairing component is p-RNA.

49. The method of claim 43 wherein at least one pairing component is CNA.

50. The method of claim 43 wherein at least one pairing component is DNA.

51. The method of claim 43 wherein at least one pairing component is RNA.

25 52. The method of claim 43 wherein at least one of the binding immunoreaction components is attached to its respective pairing component member through a covalent bond.

53. The method of claim 43 wherein at least one of the binding immunoreaction components is attached to its respective pairing component member through a

non-covalent interaction.

54. The method of claim 53 wherein the non-covalent interaction is selected from the group consisting of a biotin-streptavidin interaction and a biotin-avidin interaction.

5 55. The method of claim 43 wherein each complementary pairing component member is attached to its respective test site through a covalent bond to the permeation layer.

10 56. The method of claim 43 wherein each complementary pairing component member is attached to the test site through a non-covalent interaction with a moiety in the permeation layer.

57. The method of claim 56 wherein the non-covalent interaction is selected from the group consisting of a biotin-streptavidin interaction and a biotin-avidin interaction.

58. The method of claim 43 wherein one analyte ($I_1 C_A$) is detected.

15 59. The method of claim 43 wherein at least two analytes ($I_1 C_A, I_2 C_A$) are detected.

60. The method of claim 43 wherein at least three analytes ($I_1 C_A, I_2 C_A, I_3 C_A$) are detected.

61. The method of claim 43 wherein at least four analytes ($I_1 C_A, I_2 C_A, I_3 C_A, I_4 C_A$) are detected.

20 62. The method of claim 43 wherein at least five analytes ($I_1 C_A, I_2 C_A, I_3 C_A, I_4 C_A, I_5 C_A$) are detected.

63. The method of claim 43 wherein at least ten analytes ($I_1 C_A, \dots, I_{10} C_A$) are detected.

25 64. The method of claim 43 wherein the set of binding immunoreaction component-pairing component member complexes is contacted with the active electronic matrix array concurrently, wherein all test sites to which the binding immunoreaction component-pairing component member complexes of the set are to be bound are electronically biased concurrently, and wherein each immobilized binding immunoreaction component is immobilized with a different pairing

component.

65. The method of claim 43 wherein at least some of the set of binding immunoreaction component-pairing component member complexes are contacted with the active electronic matrix array sequentially, wherein at least some of test sites to which the binding immunoreaction component-pairing component member complexes of the set are to be bound are electronically biased sequentially, and wherein each immobilized binding immunoreaction component is immobilized with a different pairing component.

66. The method of claim 43 wherein at least some of the set of binding immunoreaction component-pairing component member complexes are contacted with the active electronic matrix array sequentially, wherein at least some of the test sites to which the binding immunoreaction component-pairing component member complexes of the set are to be bound are electronically biased sequentially, and wherein two or more immobilized binding immunoreaction component are immobilized with the same pairing component.

67. The method of claim 43 wherein the active electronic matrix array comprises at least two sets of test sites, each set comprising at least one test site comprising a complementary pairing component member for each binding immunoreaction component-pairing component member complex, and wherein in step (b) at least two sets of attached binding immunoreaction component-pairing component member complexes ($I_n C_B - P_x - P_x'$) bound to each set of test sites are created.

68. The method of claim 43 wherein the sample is incubated with the active matrix array without electronic biasing of any of the test sites on the array.

69. The method of claim 43 wherein the sample is incubated with the active matrix array while electronic biasing one or more of the test sites on the array.

70. The method of claim 69 further comprising the incubation of at least second sample containing analytes with the active electronic matrix array after incubating the first sample with the active electronic matrix array, wherein a first set of test sites comprising a first set of attached binding immunoreaction component-

pairing component member complexes is electronically biased during the incubation of the first sample with the array, and a separate set of test sites comprising another set of attached binding immunoreaction component-pairing component member complexes is electronically biased during the incubation of the each subsequent sample with the array.

5 71. The method of claim 43 further comprising contacting the active matrix array with a labeled analyte standard for at least one of the analytes to be detected, wherein each labeled analyte standard comprises a detectable moiety and a moiety selected from the group consisting of the corresponding analyte and an immunochemical analog of the corresponding analyte, wherein the detection of the presence of bound analyte in the immobilized immunoreaction complexes at the test sites in step (d) is by detecting the decrease in the amount of bound labeled analyte standard in the immobilized immunoreaction complexes at the test sites.

10 72. The method of claim 71 wherein the labeled analyte standard comprises the analyte.

15 73. The method of claim 71 wherein the analyte is an antibody, and the labeled analyte standard comprises an analyte analog comprising an isoidiotypic binding site, wherein the analyte analog is selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

20 74. The method of claim 71 wherein the analyte is an antigen, and the labeled analyte standard comprises an analyte analog comprising a structural domain selected from the group consisting of an epitope of the antigen recognized by the binding immunoreaction component which binds the analyte and a structural moiety which is anti-idiotypic to the binding site of the binding immunoreaction component which binds the analyte.

25 75. The method of claim 71 wherein the labeled analyte standard comprises a

detectable moiety selected from the group consisting of: fluorophores, chromophores, biotin, avidin, streptavidin, chemiluminescent agents, enzymes, gold particles, magnetic beads, metal chelates, radioisotopes, other antibodies, and nanoparticles.

5 76. The method of claim 71 wherein the labeled analyte standard comprises a fluorophore selected from the group consisting of: BODIPY_{630/650} X-SE, Texas Red X-SE, or BODIPY TRX-SE, Cy-dyes, fluorescein, rhodamine, phycoerythrin, Lissamine, and coumarin.

10 77. The method of claim 71 wherein the labeled analyte standard is contacted with the active matrix array before the sample is incubated with the active electronic matrix array in step (c).

78. The method of claim 71 wherein the labeled analyte standard is contacted with the active matrix array after the sample is incubated with the active electronic matrix array in step (c).

15 79. The method of claim 71 wherein the labeled analyte standard is contacted with the active matrix array while the sample is incubated with the active electronic matrix array in step (c).

80. The method of claim 71 wherein the labeled analyte standard is contacted with the active matrix without electronic biasing of any of the test sites on the array.

20 81. The method of claim 71 wherein the labeled analyte standard is contacted with the active matrix while electronically biasing one or more of the test sites on the array.

82. The method of claim 43 further comprising allowing one or more labeling immunoreaction components to bind to one or more of the analytes, wherein at least one of the labeling immunoreaction components comprises a detectable moiety, and wherein the detection of the presence of at least one bound analyte in immobilized immunoreaction complexes at the test site in step (d) is by detecting labeling immunoreaction components bound to the analyte.

25 83. The method of claim 82 wherein at least one of the labeling immunoreaction

components comprises a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

5 84. The method of claim 82 wherein at least one of the labeling immunoreaction components comprises a moiety selected from the group consisting of biotin, avidin, and streptavidin.

85. The method of claim 82 wherein at least one of the labeling immunoreaction components comprises a detectable moiety selected from the group consisting of 10 fluorophores, chromophores, biotin, avidin, streptavidin, chemiluminescent agents, enzymes, gold particles, magnetic beads, metal chelates, radioisotopes, other antibodies, and nanoparticles.

86. The method of claim 82 wherein at least one of the labeling immunoreaction components comprises a fluorophore selected from the group consisting of: 15 BODIPY_{630/650} X-SE, Texas Red X-SE, or BODIPY TRX-SE, Cy-dyes, fluorescein, rhodamine, phycoerythrin, Lissamine, and coumarin.

87. The method of claim 82 wherein at least one of the labeling immunoreaction components binds to more than one analyte in the sample.

88. The method of claim 82 wherein at least one of the labeling immunoreaction components is a dye with an affinity for one or more analytes greater than its 20 affinity for any other component of the immunoreaction complex or the permeation layer of the active electronic matrix.

89. The method of claim 82 wherein at least one of the labeling immunoreaction components is contacted with the sample prior to the incubation of the sample 25 with the active electronic matrix array in step (c).

90. The method of claim 82 wherein at least one of the labeling immunoreaction components is contacted with the sample during the incubation of the sample with the active electronic matrix array in step (c).

91. The method of claim 82 wherein at least one of the labeling immunoreaction

components is contacted with the immobilized immunoreaction complexes produced in step (c).

92. The method of claim 43 further comprising electronically biasing one or more sites containing immobilized immunoreaction complexes while contacting the at least one labeling immunoreaction component with the immobilized immunoreaction complexes.

5

93. A method for performing multiple immunological reactions on a active electronic matrix array device comprising the steps of:

- a) providing a plurality of microelectronic sites, each site having a complementary pairing component member couple thereto, the complementary pairing component members varying from site to site,
- 10 b) providing a plurality of different types of antibodies, each type of antibody being labeled with a different pairing component member, the different pairing components being complementary to the pairing components coupled to the microelectronic sites,
- c) providing at least one antigen in a sample,
- d) contacting the sample and the plurality of labeled, different types of antibodies with the pairing components at the microelectronic sites,
- 15 e) interacting the antibody-bound pairing component member and the test-site bound complementary pairing component member, and
- f) determining the sites at which an antibody coupled to the antigen coupled to the complementary pairing component is present.

20

94. The method of claim 93 wherein the antigen and plurality of labeled, different types of antibodies are provided together in a homogeneous format.

25

95. The method of claim 93 wherein the pairing component members and their corresponding complement pairing component members are p-RNA.

96. A kit for use in detecting a set one or more of analytes ($I_1 C_A, \dots, I_n C_A$) in a sample, the kit comprising a set of binding immunoreaction component-pairing component complexes ($I_1 C_B-P_1, \dots, I_n C_B-P_x$).

97. The kit of claim 96 wherein at least one of the binding immunoreaction components in the complexes of the set is an epitope bearing moiety.

98. The kit of claim 96 wherein at least one of the binding immunoreaction components in the complexes of the set is a moiety selected from the group consisting of: immunoglobulins, recombinant immunoglobulins, single-stranded engineered immunoglobulins humanized immunoglobulins, hybridized immunoglobulins, immunoglobulin derivatives, and immunoglobulin fragments.

5 99. The kit of claim 96 wherein the pairing component portions of the complexes are p-RNA.

10 100. The kit of claim 96 wherein the pairing component portions of the complexes are CNA.

101. The kit of claim 96 wherein the pairing component portions of the complexes are DNA.

102. The kit of claim 96 wherein the pairing component portions of the complexes are RNA.

15 103. The kit of claim 96 wherein at least one binding immunoreaction component is attached to its pairing component member through a covalent bond.

104. The kit of claim 96 wherein at least one binding immunoreaction component is attached to its pairing component member through a non-covalent interaction.

20 105. The kit of claim 104 wherein the non-covalent interaction is selected from the group consisting of a biotin-streptavidin interaction and a biotin-avidin interaction.

106. The kit of claim 96 further comprising a set of complementary pairing component members (P_1', \dots, P_x'), wherein the complementary pairing component members are functionalized for attachment to the test sites of an active electronic matrix device.

25 107. The kit of claim 106, wherein the complementary pairing component members are p-RNA.

108. The kit of claim 106, wherein the complementary pairing component members are CNA.
109. The kit of claim 106, wherein the complementary pairing component members are DNA.
- 5 110. The kit of claim 106, wherein the complementary pairing component members are RNA.
111. The kit of claim 96 further comprising at least one labeled analyte standard which is bound by at least one of the binding immunoreaction component-pairing component complexes in the set.
- 10 112. The kit of claim 96 further comprising at least one labeling immunoreaction component which binds to at least one of the analytes to be detected with the kit.